FILE 'REGISTRY' ENTERED AT 14:19:39 ON 15 APR 2004

=> S HYALURONIDASE/CN
L1 4 HYALURONIDASE/CN

=> D 1-4

L1 ANSWER 1 OF 4 REGISTRY COPYRIGHT 2004 ACS on STN
RN 37326-33-3 REGISTRY
CN Hyaluronoglucosaminidase (9CI) (CA INDEX NAME)
OTHER NAMES:
CN E.C. 3.2.1.34
CN E.C. 3.2.1.35
CN Hyalase
CN Hyalosidase
CN Hyaluronate 4-glycanohydrolase
CN **Hyaluronidase**
CN Hyaluronoglucosidase
CN Neopermease
MF Unspecified
CI MAN
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CABA, CAPLUS, CHEMCATS, CHEMLIST, CIN, CSCHEM, DIOGENES, EMBASE, IMSDRUGNEWS, IMSRESEARCH, IPA, MEDLINE, MSDS-OHS, PROMT, TOXCENTER, USAN, USPAT2, USPATFULL
Other Sources: EINECS**, WHO
(**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
487 REFERENCES IN FILE CA (1907 TO DATE)
4 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
491 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 2 OF 4 REGISTRY COPYRIGHT 2004 ACS on STN
RN 37288-34-9 REGISTRY
CN Hyaluronoglucuronidase (9CI) (CA INDEX NAME)
OTHER NAMES:
CN E.C. 3.2.1.36
CN Glucuronoglycosaminoglycan hyaluronate lyase
CN Hyaluronate 3-glycanohydrolase
CN **Hyaluronidase**
CN Manillase
MF Unspecified
CI COM, MAN
LC STN Files: ADISNEWS, AGRICOLA, BIOBUSINESS, BIOSIS, CA, CABA, CAPLUS, CIN, DIOGENES, IFICDB, IFIPAT, IFIUDB, MEDLINE, PROMT, TOXCENTER, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
37 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
37 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 3 OF 4 REGISTRY COPYRIGHT 2004 ACS on STN
RN 37259-53-3 REGISTRY
CN Lyase, hyaluronate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN E.C. 4.2.2.1
CN E.C. 4.2.99.1
CN Glucuronoglycosaminoglycan lyase

CN Hyaluronate lyase
CN **Hyaluronidase**
CN Lyase, glucuronoglycosaminoglycan
MF Unspecified
CI MAN
LC STN Files: ADISNEWS, AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CHEMLIST, CIN, DIOGENES, EMBASE, IPA, MEDLINE, PROMT, TOXCENTER, USPATFULL
Other Sources: EINECS**
(**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
202 REFERENCES IN FILE CA (1907 TO DATE)
11 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
203 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 4 OF 4 REGISTRY COPYRIGHT 2004 ACS on STN
RN 9001-54-1 REGISTRY
CN **Hyaluronidase (9CI)** (CA INDEX NAME)
OTHER NAMES:
CN Alidase
CN Diffusin
CN Diffusing factor
CN Enzodase
CN Hyadase
CN Hyalidase
CN Hyason
CN Hyazyme
CN Hylase
CN Infiltrase
CN Jalovis
CN Kinaden
CN Kinetin
CN Kinetin (enzyme)
CN Kinetin-Schering
CN Lidase
CN Luronase
CN Mucinase
CN Receptor-destroying mucinase
CN Rondase
CN Ronidase
CN Spreading factor
CN Thiomucase
CN Unidasa
CN Uterolidase
CN Wydase
DR 9013-16-5, 9013-54-1, 9013-76-7, 9013-97-2, 9037-26-7
MF Unspecified
CI COM, MAN
LC STN Files: ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CASREACT, CHEMCATS, CHEMLIST, CIN, CSCHEM, DDFU, DIOGENES, DRUGU, EMBASE, IFICDB, IFIPAT, IFIUDB, IPA, MEDLINE, MRCK*, MSDS-OHS, NAPRALERT, NIOSHTIC, PHAR, PIRA, PROMT, TOXCENTER, USAN, USPAT2, USPATFULL
(*File contains numerically searchable property data)
Other Sources: EINECS**, TSCA**, WHO
(**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
2361 REFERENCES IN FILE CA (1907 TO DATE)

24 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
2363 REFERENCES IN FILE CAPLUS (1907 TO DATE)

FILE 'CAPLUS' ENTERED AT 14:20:45 ON 15 APR 2004

=> S L1;S HYALURONIDASE;S L1 OR L2;S HIRUDINARIA;S MANILLENSIS
L2 3048 L1

7465 HYALURONIDASE
1886 HYALURONIDASES
L3 7670 HYALURONIDASE
(HYALURONIDASE OR HYALURONIDASES)

3048 L1
L4 3048 L1 OR L2

L5 36 HIRUDINARIA

L6 41 MANILLENSIS

=> S L4 AND L5
L7 2 L4 AND L5

=> S L7 AND L6
L8 1 L7 AND L6

=> D L7 1-2 CBIB ABS

L7 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
2000:900820 Document No. 134:67185 Protein and cDNA sequences of a novel **hirudinaria** manillensis hyaluronidase and therapeutic uses thereof. Kordowicz, Maria; Gussow, Detlef; Hofmann, Uwe; Pacuszka, Tadeusz; Gardas, Andrzej (Merck Patent Gmbh, Germany). PCT Int. Appl. WO 2000077221 A1 20001221, 71 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-EP5181 20000606. PRIORITY: EP 1999-111468 19990612.

AB The present invention relates to the isolation, purification and characterization of a hyaluronidase which derives from the tropical leech **Hirudinaria** manillensis. Therefore, according to this invention, the enzyme was called "manillase". The invention is furthermore concerned with the recombinant method of production of manillase which includes the disclosure of DNA and amino acid sequences as well as of expression vectors and host systems. Finally, the invention relates to the use of manillase for therapeutic purposes, for example, for the treatment of myocardial diseases, thrombotic events and tumors.

L7 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
1987:531241 Document No. 107:131241 The saliva of the medicinal leech Hirudo medicinalis. I. Biochemical characterization of the high molecular

weight fraction. Rigbi, Meir; Levy, Haim; Iraqi, Fuad; Teitelbaum, Mira; Orevi, Miriam; Alajoutsijarvi, Arja; Horovitz, Amnon; Galun, Rachel (Inst. Life Sci., Hebrew Univ. Jerusalem, Jerusalem, 91904, Israel). Comparative Biochemistry and Physiology, Part B: Biochemistry & Molecular Biology, 87B(3), 567-73 (English) 1987. CODEN: CBPBB8. ISSN: 0305-0491.

AB A method is described for obtaining dilute H. medicinalis saliva by feeding leeches through a membrane on arginine/saline and squeezing them immediately after from the posterior end forwards. The process can be repeated at intervals. Yields are considerably higher than those from salivary gland exts. Hirudo Saliva contains hirudin, eglin, hyaluronidase, collagenase, and apyrase. Leech collagenase and apyrase are reported here for the 1st time. On gel filtration of lyophilized saliva, the activity peaks are well defined. Approx. mol. wts. are determined Apyrase appears in 2 forms with optimum activity around pH 7.5. Collagenase is identified as belonging to the mammalian type.

=> S L5(W)L6
L9 29 L5(W)L6

=> S L9 NOT L7
L10 28 L9 NOT L7

=> D 1-28 TI

=> D L10 14,18,19,23,25,27 CBIB ABS

L10 ANSWER 14 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN
1993:598023 Document No. 119:198023 The complete amino acid sequence of a hirudin variant from the leech **Hirudinaria manillensis**. Electricwala, A.; Hartwell, R.; Scawen, M. D.; Atkinson, T. (Cent. Appl. Microbiol. Res., PHLS, Salisbury, SP4 0JG, UK). Journal of Protein Chemistry, 12(3), 365-70 (English) 1993. CODEN: JPCHD2. ISSN: 0277-8033.

AB Unlike the European leech Hirudo medicinalis, the Asian jawed leech **Hirudinaria manillensis** is specialized for feeding on mammalian blood. In the salivary glands of both these leeches, there is a potent inhibitor of thrombin, called hirudin, which acts as an anticoagulant. The authors have reported previously the isolation and purification of a variant of hirudin, called bufrudin, from the head portions of Hirudinaria. In the present study, the complete amino acid sequence of bufrudin was determined by automated Edman degradation of peptide fragments generated after cleavage of protein with trypsin or thermolysin. Comparison of the primary structure of bufrudin, with hirudin HV1, show about 70% sequence identity with deletion of two amino acids, but the key amino acids at the C-terminus, involved in the inhibition of thrombin, are conserved. However, similar sequence comparison of bufrudin with hirullin P18, a hirudin variant isolated from same leech species but from whole leech, instead of heads, reveals even less sequence identity of about 60%. From the amino acid sequence, it is suggested that the conformation of the C-terminal portion of bufrudin may be significantly different from hirullin P18, but similar to hirudin HV1, upon its interaction with thrombin. These results indicate that, as with Hirudo leech, various isoforms of hirudin also exist in Hirudinaria leech, with a significant change occurring in the structure of the mol. during the evolution of leeches.

L10 ANSWER 18 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN
1993:463043 Document No. 119:63043 Anticoagulant peptides from the leech **Hirudinaria manillensis**. Sarmientos, Paolo; De Taxis du

Poet, Philippe; Nitti, Giampaolo; Scacheri, Emanuela (Farmitalia Carlo Erba S.r.l., Italy). Eur. Pat. Appl. EP 501821 A2 19920902, 60 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, PT, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1992-301721 19920228. PRIORITY: GB 1991-4260 19910228; GB 1991-19954 19910918.

AB Thrombin-inhibiting peptides distinct from the hirudins of Hirudo medicinalis are obtained from **Hirudinaria manillensis** and characterized and synthetic genes encoding them synthesized and expressed in heterologous hosts. The proteins have uses similar to those of hirudins and may present some advantages. The proteins were purified from aqueous acetone exts. of leech heads by ion-exchange, thrombin-affinity, and reversed-phase chromatog. and sequenced. A synthetic gene for 1 of the peptides was prepared with a codon usage optimized for expression in Escherichia coli. Secretory expression of the gene was achieved using the signal sequence of the ompA gene.

L10 ANSWER 19 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN

1992:146333 Document No. 116:146333 Primary structure and function of novel O-glycosylated hirudins from the leech **Hirudinaria manillensis**. Steiner, Verena; Knecht, Rene; Boernsen, K. Olaf; Gassmann, Ernst; Stone, Stuart R.; Raschdorf, Fritz; Schlaeppi, Jean Marc; Maschler, Reinhard (Ciba-Geigy Ltd., Basel, CH-4002, Switz.). Biochemistry, 31(8), 2294-8 (English) 1992. CODEN: BICHAW. ISSN: 0006-2960.

AB Hirudin from the leech Hirudo medicinalis is a most powerful anticoagulant, and many isoforms have been described. In the present work, the primary structure of two hirudins from the leech **Hirudinaria manillensis** has been elucidated. The antithrombotic activity is similar to that of H. medicinalis hirudins although the sequence identity is below 60%. Surprisingly, the hirudins were found to be glycosylated at one site. Sugar anal. after methanolysis yielded fucose, galactose, and N-acetylgalactosamine. These results combined with data from matrix-assisted laser desorption ionization mass spectrometry, plasma desorption mass spectrometry, capillary zone electrophoresis, and lectin-binding tests indicate that the sequence is Fuc-Galβ1-3GalNAc-(O-threonine). This structure shows an interesting similarity to human blood group H determinants.

L10 ANSWER 23 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN

1991:36001 Document No. 114:36001 Proteins and peptides from **Hirudinaria manillensis** with antithrombin activity. Sawyer, Roy; Powell-Jones, Christopher; Atkinson, Anthony; Electricwala, Asgar (Biopharm (UK) Ltd., UK). PCT Int. Appl. WO 9005143 A1 19900517, 25 pp. DESIGNATED STATES: W: AU, BG, BR, DK, FI, GB, HU, JP, KP, KR, LK, MC, NO, RO, SU, US; RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1989-GB1345 19891113. PRIORITY: GB 1988-26428 19881111.

AB A polypeptide X-Y-Tyr-Thr-Asp-Cys-Thr-Glu-Ser-Gly-Gln-Asn-Tyr-Cys-Leu-Cys-Val-Gly-Ser-Asn-Val-Cys-Gly-Glu-Gly-Asp-Asn-Cys-Asn-D-Gln-Leu-Ser-Ser-Ser-Gly-Asn-Gln-Cys-Val-E-Gly-Glu-Gly-Thr-Pro-F-Pro-Gln-Ser-Gln-Thr-Glu-Gly- Asp-Phe-Glu-Glu-Ile-Pro-Asp-Glu-Z-Ile-Lys (I; X, Y, Z = any amino acid residue; D = Cys, Pro; E = Glu, Asp, His; F = Asp, Lys, Trp) and, pharmaceutically acceptable salts, derivs., and bioprecursors thereof are provided. Also provided is the peptide Gly-Asp-Phe-Glu-Glu-Ile-Pro-Asp- Glu-Z-Ile-Lys (Z as above) which specifically inhibits thrombin, or a pharmaceutically acceptable salt, derivative, or bioprecursor thereof. I was acetone-extracted from H. manillensis, further purified, and sequenced. The activity of the antithrombin species of the invention was not neutralized by a high concentration of neutralizing monoclonal antibodies specific for hirudin. The antithrombin species of the invention and hirudin did, however, have similar

partial thromboplastic times for equivalent doses, suggesting similar anticoagulant properties. Comparison of I and the hirudin sequence indicated an approx. 62% homol.

L10 ANSWER 25 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN
1991:2852 Document No. 114:2852 Isolation and purification of novel hirudins from the leech **Hirudinaria manillensis** by high-performance liquid chromatography. Steiner, Verena; Knecht, Rene; Gruetter, Markus; Raschdorf, Fritz; Gassmann, Ernst; Maschler, Reinhard (Pharm. Res. Lab., Ciba-Geigy Ltd., Basel, CH-4002, Switz.). Journal of Chromatography, 530(2), 273-82 (English) 1990. CODEN: JOCRAM. ISSN: 0021-9673.

AB The isolation and purification of novel hirudins from a crude extract of the leech H. manillensis and their anal. characterization are reported. Initial purification by gel permeation chromatog. on Sephadex G50 and anion-exchange chromatog. on Q Sepharose fast-flow removed most contaminants and yielded a highly active extract Two isohirudins (designated hirudin P6 and P18) were isolated and purified by successive reverse-phase HPLC on silica-based stationary phases and anion-exchange chromatog. on Mono Q. The final products were characterized by reversed-phase HPLC, 252Cf plasma desorption time-of-flight mass spectrometry, and capillary zone electrophoresis. The mol. masses determined by 252Cf plasma desorption mass spectrometry were 7416 dalton for hirudin P6 and 7199 dalton for hirudin P18.

L10 ANSWER 27 OF 28 CAPLUS COPYRIGHT 2004 ACS on STN
1986:621743 Document No. 105:221743 Hyaluronidase its isolation, pharmaceutical and veterinary use. Sawyer, Roy Thomas; Edwards, Jeffrey (Biopharm (UK) Ltd., UK). Eur. Pat. Appl. EP 193330 A2 19860903, 21 pp. DESIGNATED STATES: R: DE, FR, GB, IT. (English). CODEN: EPXXDW. APPLICATION: EP 1986-301092 19860217. PRIORITY: GB 1985-4025 19850216.

AB Hyaluronidase, which is a hyaluronic acid-specific endoglucuronidase, having a mol. weight of .apprx.28,500 in nonreduced form, is derived from buffalo leeches (i.e., leeches of the sub-family Hirudinariinae, such as the species H. manillensis or Poecilobdella granulosa). Hyaluronidase, which cleaves hyaluronic acid, but not chondroitin, chondroitin-4-sulfate, chondroitin-6-sulfate, or heparin, is considerably more stable at high temps. and extremes of pH than known leech hyaluronidase. It has a wide range of uses where breakdown of hyaluronic acid is required; of particular interest is in pharmaceutical or veterinary formulations, either as an active agent or a spreading or percutaneous factor. Hyaluronidase is useful for stimulated flow of physiol. fluids in the eye (for example, in the treatment of glaucoma).

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=> E KORDOWICA/AU
=> S E7,E8
             3 "KORDOWICZ M"/AU
            20 "KORDOWICZ MARIA"/AU
L11         23 ("KORDOWICZ M"/AU OR "KORDOWICZ MARIA"/AU)

=> E GUSSOW/AU
=> S E4-E8
             1 "GUSSOW D"/AU
             1 "GUSSOW D H"/AU
            12 "GUSSOW DETLEF"/AU
             3 "GUSSOW DETLEF H"/AU
             1 "GUSSOW DETLEV"/AU
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L12          18 ("GUSSOW D"/AU OR "GUSSOW D H"/AU OR "GUSSOW DETLEF"/AU OR "GUSS
                OW DETLEF H"/AU OR "GUSSOW DETLEV"/AU)

=> E HOFMANN U/AU
=> S E3,E15,E16,E17
            186 "HOFMANN U"/AU
             50 "HOFMANN UTE"/AU
             35 "HOFMANN UWE"/AU
              1 "HOFMANN UWE R"/AU
L13         272 ("HOFMANN U"/AU OR "HOFMANN UTE"/AU OR "HOFMANN UWE"/AU OR "HOFM
                ANN UWE R"/AU)

=> E PACUSZKA/AU
=> S E4,E5
              3 "PACUSZKA T"/AU
             33 "PACUSZKA TADEUSZ"/AU
L14          36 ("PACUSZKA T"/AU OR "PACUSZKA TADEUSZ"/AU)

=> E GARDAS/AU
=> S E4-E7
              7 "GARDAS A"/AU
              1 "GARDAS ANDREJ"/AU
              2 "GARDAS ANDREZEJ"/AU
             39 "GARDAS ANDRZEJ"/AU
L15          49 ("GARDAS A"/AU OR "GARDAS ANDREJ"/AU OR "GARDAS ANDREZEJ"/AU OR
                "GARDAS ANDRZEJ"/AU)

=> S L11,L12,L13,L14,L15
L16         392 (L11 OR L12 OR L13 OR L14 OR L15)

=> S L16 AND L4
L17           1 L16 AND L4

=> S L17 NOT L7
L18           0 L17 NOT L7
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FILE 'REGISTRY' ENTERED AT 15:00:37 ON 15 APR 2004

=> S HYALURONIDASE/CN
L1 4 HYALURONIDASE/CN

FILE 'CAPLUS' ENTERED AT 15:00:56 ON 15 APR 2004

=> S L1;S HYALURONIDASE;S HIRUDO;S MANILLENSIS
L2 3048 L1

7465 HYALURONIDASE
1886 HYALURONIDASES
L3 7670 HYALURONIDASE
(HYALURONIDASE OR HYALURONIDASES)

L4 863 HIRUDO

L5 41 MANILLENSIS

=> S L2,L3
L6 8009 (L2 OR L3)

=> S HIRUDINARIA
L7 36 HIRUDINARIA

=> S L4 AND L6;S L7 AND L6
L8 6 L4 AND L6

L9 4 L7 AND L6

=> S L8,L9
L10 9 (L8 OR L9)

=> D 1-9 CBIB ABS

L10 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
2004:74592 Document No. 140:212899 Mechanism of Activation of Human Heparanase Investigated by Protein Engineering. Nardella, Caterina; Lahm, Armin; Pallaoro, Michele; Brunetti, Mirko; Vannini, Alessandro; Steinkuehler, Christian (Department of Biochemistry, IRBM/Merck Research Laboratories, Pomezia, 30600, Italy). Biochemistry, 43(7), 1862-1873 (English) 2004. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB The aim of this study was to investigate the mechanism of activation of human heparanase, a key player in heparan sulfate degradation, thought to be involved in normal and pathol. cell migration processes. Active heparanase arises as a product of a series of proteolytic processing events. Upon removal of the signal peptide, the resulting, poorly active 65 kDa species undergoes the excision of an intervening 6 kDa fragment generating an 8 kDa polypeptide and a 50 kDa polypeptide, forming the fully active heterodimer. By engineering of tobacco etch virus protease cleavage sites at the N- and C-terminal junctions of the 6 kDa fragment, we were able to reproduce the proteolytic activation of heparanase in vitro using purified components, showing that cleavage at both sites leads to activation in the absence of addnl. factors. On the basis of multiple-sequence alignment of the N-terminal fragment, we conclude that the first β/α/β element of the postulated TIM

barrel fold is contributed by the 8 kDa subunit and that the excised 6 kDa fragment connects the second β-strand and the second α-helix of the barrel. Substituting the 6 kDa fragment with the topol. equivalent loop from **Hirudinaria** manillensis **hyaluronidase** or connecting the 8 and 50 kDa fragments with a spacer of three glycine-serine pairs resulted in constitutively active, single-chain heparanases which were comparable to the processed, heterodimeric enzyme with regard to specific activity, chromatog. profile of hydrolysis products, complete inhibition at NaCl concns. above 600 mM, a pH optimum of pH .apprx.5, and inhibition by heparin with IC50s of 0.9-1.5 ng/μL. We conclude that (1) the heparanase heterodimer (α/β)8-TIM barrel fold is contributed by both 8 and 50 kDa subunits with the 6 kDa connecting fragment leading to inhibition of heparanase by possibly obstructing access to the active site, (2) proteolytic excision of the 6 kDa fragment is necessary and sufficient for heparanase activation, and (3) our findings open the way to the production of recombinant, constitutively active single-chain heparanase for structural studies and for the identification of inhibitors.

L10 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

2000:900820 Document No. 134:67185 Protein and cDNA sequences of a novel **hirudinaria** manillensis **hyaluronidase** and therapeutic uses thereof. Kordowicz, Maria; Gussow, Detlef; Hofmann, Uwe; Pacuszka, Tadeusz; Gardas, Andrzej (Merck Patent Gmbh, Germany). PCT Int. Appl. WO 2000077221 A1 20001221, 71 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-EP5181 20000606. PRIORITY: EP 1999-111468 19990612.

AB The present invention relates to the isolation, purification and characterization of a **hyaluronidase** which derives from the tropical leech **Hirudinaria** manillensis. Therefore, according to this invention, the enzyme was called "manillase". The invention is furthermore concerned with the recombinant method of production of manillase which includes the disclosure of DNA and amino acid sequences as well as of expression vectors and host systems. Finally, the invention relates to the use of manillase for therapeutic purposes, for example, for the treatment of myocardial diseases, thrombotic events and tumors.

L10 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

2000:510931 Document No. 133:329303 Biological activity and pharmacological properties of anticoagulant complex (hirudin, plasma kallikrein inhibitor, prostaglandin) from **Hirudo** medicinalis. Nikonov, G. I.; Titova, E. A.; Seleznev, K. G. (BIOKON, Medical Research-and-Production Company, Moscow Region, Russia). Bulletin of Experimental Biology and Medicine (Translation of Byulleten Eksperimental'noi Biologii i Meditsiny), Volume Date 1999, 128(12), 1244-1247 (English) 2000. CODEN: BEXBAN. ISSN: 0007-4888. Publisher: Consultants Bureau.

AB Antiprocoagulant complex isolated from lyophilized medicinal leeches exerted pronounced antithrombotic, thrombolytic, and hypotensive effects in exptl. animals after i.v. injection and showed antithrombotic activity after oral administration in combination with hirudone, the source of **hyaluronidase** and inhibitors of digestive proteolytic enzymes. The antiprocoagulant complex can be used as a specific medicinal preparation

L10 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
1999:791884 Document No. 132:61820 **Hyaluronidase** activity in leeches (Hirudinea). Hovingh, Peter; Linker, Alfred (Veterans Administration Hospital, Salt Lake City, UT, 84148, USA). Comparative Biochemistry and Physiology, Part B: Biochemistry & Molecular Biology, 124B(3), 319-326 (English) 1999. CODEN: CBPBB8. ISSN: 0305-0491. Publisher: Elsevier Science Inc..

AB The leech hyaluronoglucuronidase (**hyaluronidase** I) was identified in Erpobdellidae (Nephelopsis obscura and Erpobdella punctata) and Glossiphoniidae (Desserobdella picta) and historically described from Hirudinidae (**Hirudo** medicinalis). A 2nd leech **hyaluronidase** (**hyaluronidase** II) which hydrolyzed only a few bonds to form hyaluronan oligosaccharides >6500 Da, was found in Glossiphoniidae (Helobdella stagnalis, Glossiphonia complanata, Placobdella ornata, and Theromyzon sp.) and in Haemopidae (Haemopis marmorata). The distribution of the 2 **hyaluronidases** in leech occurred in both orders (Arhynchobdellida and Rhynchobdellida) and in macrophagous and hematophagous feeding types whereas the liquidosomatophagous leeches only had **hyaluronidase** II.

L10 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
1987:549979 Document No. 107:149979 A comparison of the properties of the **hyaluronidases** from a temperate and a tropical species of leech. Budds, Michele; Edwards, Jeffrey; Olavesen, Anthony H.; Gacesa, Peter (Dep. Biochem., Univ. Coll., Cardiff, CF1 1XL, UK). Comparative Biochemistry and Physiology, Part B: Biochemistry & Molecular Biology, 87B(3), 497-500 (English) 1987. CODEN: CBPBB8. ISSN: 0305-0491.

AB Some properties of a **hyaluronidase** preparation (Orgelase) from a tropical Asian species of leech (Sanguisoga granulosa) were compared to the enzyme from **Hirudo** medicinalis. Both enzymes had an endo-β-glucuronidase mode of action and were specific for hyaluronic acid. The enzyme from the tropical leech was considerably more heat stable and was active over a broader pH range than that from **Hirudo**. Both enzymes were inhibited by Hg2+, but were unaffected by saccharo-1,4-lactone or NaN3.

L10 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
1987:531241 Document No. 107:131241 The saliva of the medicinal leech **Hirudo** medicinalis. I. Biochemical characterization of the high molecular weight fraction. Rigbi, Meir; Levy, Haim; Iraqi, Fuad; Teitelbaum, Mira; Orevi, Miriam; Alajoutsijarvi, Arja; Horovitz, Amnon; Galun, Rachel (Inst. Life Sci., Hebrew Univ. Jerusalem, Jerusalem, 91904, Israel). Comparative Biochemistry and Physiology, Part B: Biochemistry & Molecular Biology, 87B(3), 567-73 (English) 1987. CODEN: CBPBB8. ISSN: 0305-0491.

AB A method is described for obtaining dilute H. medicinalis saliva by feeding leeches through a membrane on arginine/saline and squeezing them immediately after from the posterior end forwards. The process can be repeated at intervals. Yields are considerably higher than those from salivary gland exts. **Hirudo** Saliva contains hirudin, eglin, **hyaluronidase**, collagenase, and apyrase. Leech collagenase and apyrase are reported here for the 1st time. On gel filtration of lyophilized saliva, the activity peaks are well defined. Approx. mol. wts. are determined Apyrase appears in 2 forms with optimum activity around pH 7.5. Collagenase is identified as belonging to the mammalian type.

L10 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
1986:621743 Document No. 105:221743 **Hyaluronidase** its isolation,

pharmaceutical and veterinary use. Sawyer, Roy Thomas; Edwards, Jeffrey (Biopharm (UK) Ltd., UK). Eur. Pat. Appl. EP 193330 A2 19860903, 21 pp. DESIGNATED STATES: R: DE, FR, GB, IT. (English). CODEN: EPXXDW. APPLICATION: EP 1986-301092 19860217. PRIORITY: GB 1985-4025 19850216.

AB **Hyaluronidase**, which is a hyaluronic acid-specific endoglucuronidase, having a mol. weight of .apprx.28,500 in nonreduced form, is derived from buffalo leeches (i.e., leeches of the sub-family Hirudinariinae, such as the species H. manillensis or Poecilobdella granulosa). **Hyaluronidase**, which cleaves hyaluronic acid, but not chondroitin, chondroitin-4-sulfate, chondroitin-6-sulfate, or heparin, is considerably more stable at high temps. and extremes of pH than known leech **hyaluronidase**. It has a wide range of uses where breakdown of hyaluronic acid is required; of particular interest is in pharmaceutical or veterinary formulations, either as an active agent or a spreading or percutaneous factor. **Hyaluronidase** is useful for stimulated flow of physiol. fluids in the eye (for example, in the treatment of glaucoma).

L10 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

1960:98970 Document No. 54:98970 Original Reference No. 54:18809i,18810a Production of hyaluronate oligosaccharides by leech **hyaluronidase** and alkali. Linker, Alfred; Meyer, Karl; Hoffman, Philip (Columbia Univ.). Journal of Biological Chemistry, 235, 924-7 (Unavailable) 1960. CODEN: JBCHA3. ISSN: 0021-9258.

AB cf. CA 52, 4036h. The **hyaluronidase** of the medicinal leech is an endoglucuronidase which hydrolyzes hyaluronic acid to oligosaccharides which have the uronic acid moiety on the free reducing end. Comparison compds., also having a uronic acid reducing end, were prepared by the action of lime water on oligosaccharides obtained from hyaluronate by testicular **hyaluronidase**. The leech enzyme, although a β-glucuronidase, does not act on chondroitin or chondroitin sulfate, which contain glucuronic acid, but differ in the amino sugar from hyaluronate. The **hyaluronidase** of the leech is the most specific enzyme known for the identification of hyaluronic acid.

L10 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN

1958:22308 Document No. 52:22308 Original Reference No. 52:4036g-i,4037a-b The **hyaluronidase** of the leech: an endoglucuronidase. Linker, Alfred; Hoffman, Philip; Meyer, Karl (Columbia Univ. Coll., New York, NY). Nature (London, United Kingdom), 180, 810-11 (Unavailable) 1957. CODEN: NATUAS. ISSN: 0028-0836.

AB A type of **hyaluronidase** is described which has thus far been found only in exts. of **Hirudo** medicinalis and which hydrolyzes the endoglucuronidic linkages of hyaluronic acid. Paper chromatograms of products of the 24-hr. digests of sodium hyaluronate by the leech enzyme and by testicular **hyaluronidase** showed a similar distribution of ogligosaccharides. The leech digest products, however, had slower mobilities and also differed markedly from those obtained by microbial **hyaluronidase**. The major fraction of leech oligosaccharides was isolated by ion-exchange chromatography. On paper, it produced a single spot with a slightly slower mobility than testicular tetrasaccharide. The testis enzyme, leech enzyme, and reduced leech tetrasaccharide were analyzed for percentage of hexosamine, uronic acid, acetylglucosamine color, and reducing sugar. From this analysis and from general properties it was concluded that leech oligosaccharide, in contrast to the testicular tetra-accharide, is a tetrasaccharide having a uronic acid at the reducing end group. This indicates that the leech enzyme hydrolyzes endoglucuronic bonds while testicular and microbial **hyaluronidases** split endohexosaminidic bonds. Confirmation of the structure of the leech oligosaccharides has been obtained by the action of weak alkali on various oligosaccharide fractions. The compds. obtained by leech **hyaluronidase** and by the combination of alkaline and enzymic degradation represent a new series of oligosaccharides derived from hyaluronic acid. It is now possible to prepare oligosaccharides with any combination of

reducing and non-reducing end groups.

	Hits	L #	Search Text	DBs
1	13	L1	HIRUDINARIA	USPAT ; US-PG PUB
2	15	L2	MANILLENSIS	USPAT ; US-PG PUB
3	2576	L3	HYALURONIDASE	USPAT ; US-PG PUB
4	2	L4	L1 AND L3	USPAT ; US-PG PUB
5	0	L6	L5 NOT L1	USPAT ; US-PG PUB
6	13	L5	L1 ADJ L2	USPAT ; US-PG PUB
7	203	L7	HIRUDO	USPAT ; US-PG PUB
8	3	L8	L7 ADJ L2	USPAT ; US-PG PUB